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Ilska-Warner, J; Haskell, MJ; Blott, SC; Sanchez-Molano, E; Polgar, Z; Lofgren, SE; Clements, DN; Wiener, P

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Joanna Ilska-Warner[1]
Marie J. Haskell[1]
Sarah C. Blott[2]
Enrique Sánchez-Molano[3]
Zita Polgar[3]
Sarah E. Lofgren[4]
Dylan N. Clements[3]
Pamela Wiener[3]

1. Scotland's Rural College, Edinburgh, Scotland, UK
2. University of Nottingham, Sutton Bonington, England, UK
3. Roslin Institute, University of Edinburgh, Easter Bush, Scotland, UK
4. Youth Science Institute, Los Gatos, California, USA

Abstract

Personality or individual consistency in behavioural responsiveness to stimuli and situations, is recognized in a wide range of animal species, including dogs. These traits are important for determining how well a dog fits its role (e.g. as pet or working dog) and can also influence the dog's psychological well-being. The distinct behavioural characteristics of individual dog breeds suggest a strong genetic component to personality in this species and there is also evidence for within-breed variation. However, it is a challenge to gather sufficiently large datasets to dissect the genetic basis of complex traits such as behaviour, which are both time-consuming and logistically difficult to measure, and known to be influenced by non-genetic factors. In this study, we exploited the knowledge that owners have of their own dogs to generate a large dataset of 12 personality traits in Labrador Retrievers, the most popular breed in the UK and various other countries. While accounting for key environmental factors, we demonstrate that genetic variance can be detected for dog personality traits assessed using questionnaire data. We identified substantial genetic variance for several traits, including fetching tendency and fear of loud noises, while other traits, such as owner-directed aggression, revealed negligibly small heritabilities. For comparison, an alternative set of 14 traits developed in previous studies were also analysed; differences between the heritabilities of corresponding traits in the two sets indicate that the method of grouping questionnaire data into behavioural factors may influence estimates of heritability. Genomic analyses indicated that these traits are mainly polygenic, such that individual genomic regions have small effects, and suggested chromosomal associations for eight of the traits. Our results demonstrate that dissection of genetic and non-genetic factors that influence dog personality traits can be facilitated using data provided by owners.

Author summary

Unravelling the factors influencing complex biological traits is one of the major goals of modern biology. Behavioural traits are among the most challenging due to the recognised influences of both genetic and non-genetic factors and equally, to the difficulties and costs of assembling sufficiently large sample sizes to provide reasonable statistical power. However, these traits are also among the most interesting in that they are integral to distinguishing species, breeds and individuals from each other. By exploiting the knowledge that dog owners have of their own dogs' behaviour, a large dataset was generated, suitable for genetic investigation. We demonstrated a substantial genetic component associated with a range of personality characteristics assessed using a standard dog behaviour questionnaire and associations with chromosomal regions were suggested for several of the traits.

Introduction

The distinct behavioural predispositions of individual dog breeds clearly indicate a strong genetic component to dog personality (understood as the individual consistency in behavioural responsiveness to stimuli and situations; [1]), further strengthened by estimates of substantial within-breed genetic variance found for a variety of behavioural traits across studies.

In the past, the majority of dog behaviour studies were carried out on working dogs and used standardized tests, where the effects of the environment at the time of the test could be clearly characterized. These standardized tests in controlled environments provide estimates of moderate heritability for some tested behaviours, e.g. heritability of "gun shyness" has been estimated at 0.56 (SE 0.09) [2]. However, the majority of the reported heritability estimates for these traits fall below 0.4 (e.g. [2-5]), with various management and lifestyle factors being shown to affect behaviour (e.g. training practices, [6]). Thus large datasets are required for accurate decomposition of the variance in these traits into genetic and non-genetic components. Generating such datasets requires substantial infrastructure which, in practice, may be unattainable for most pet dog populations. Thus, even though personality traits are extremely important for the well-being of both the dog and its owner, their heritabilities for pet dogs are still largely unknown.

Genomic methodologies like GWAS that assess markers across the genome have been used to determine associations between traits and particular genetic variants. However, substantial datasets are required to identify genomic associations or to use genomic prediction techniques when a large number of small genetic effects are involved, as is expected to be the case for behavioural traits [7]. As a result, few genomic analyses have been applied to dog behaviour traits so far and thus, little is known about the genetic architecture or the individual genes involved. Variation in a few functional candidate genes (e.g. *DRD4, TH, OXTR, SLC6A*) has been shown to be associated with behaviour in dogs ([8-11]). However, these detected associations are only a starting point in the process of understanding the molecular genetic basis of dog behaviour.

Thus, the size of available datasets is a limiting factor to the dissection of the variance components of behavioural traits, as well as to the characterisation of their genetic architecture. An alternative approach to using data from standardised tests would be to exploit the knowledge that pet owners and dog breeders have of their own dogs in everyday

situations, in order to accumulate large datasets suitable for dissection of behavioural traits. The size of these datasets could then overcome the lack of standardised assessment and at the same time, avoid possible interactions between the behaviour and the somewhat artificial conditions of the test environment.

A survey-based approach has been now utilized in a number of studies on dog behaviour, where the dog owner's answers to validated questionnaires, such as Canine Behavioral Assessment and Research Questionnaire (C-BARQ), were used to assess the personality traits of the dog. C-BARQ was developed at the University of Pennsylvania originally as a method for evaluating and predicting the success of guide dogs [12]. The reliability and validity of C-BARQ has been shown by the developers of the method and others (e.g. [13]) and subsequently, it has been applied in studies of dog behaviour by various groups (e.g. [14, 15]). The C-BARQ survey contains 101 questions regarding the dog's behavioural response to various situations, with answers marked on a 5-step scale. The particular items of the C-BARQ questionnaires are then typically grouped into factors describing a personality trait. In most studies (e.g. [16, 17]), the grouping and number of resulting traits are largely based on the definitions derived by the developers of the questionnaire [18, 19], who used factor analysis to define 11 (and later, 14) behavioural traits. In a previous study of Labrador Retrievers, we used multivariate statistical techniques to define 12 personality traits from C-BARQ data [20], some of which overlapped the previous grouping while others were novel.

In this paper we used quantitative genetic and genomic approaches to investigate the genetic contribution to everyday life behaviour in the Labrador Retriever breed.

Methods

Personality trait characterisation

The data used in the study were a subset of a larger study on genetics of complex traits in dogs, and consisted of owner-supplied responses to C-BARQ as well as a separate demographic questionnaire. The dataset was limited to UK Kennel Club-registered Labrador Retrievers. We previously applied a combination of Principal Components Analysis and correlation structure to derive 12 behaviour traits (subsequently referred to as "SetA traits"): Agitated when Ignored (Agitated), Attention-seeking (Attention), Barking Tendency (Barking), Excitability, Fetching, Human and Object Fear (HOFear), Noise Fear (NoiseFear),

Non-owner-directed Aggression (NOAggression), Owner-directed Aggression (OAggression), Separation Anxiety (SepAnxiety), Trainability and Unusual Behaviour (Unusual) [20]. The 12 trait values were calculated as averages of the responses observed in each associated group, where the number of questions in the group ranged from 1 (Barking, Fetching) to 20 (Unusual) (Supplementary Table 3 in [20]). The final dataset used in the current analyses included 1,975 animals. The numbers of observations and the range of scores observed for each of the SetA traits are presented in Table 1. For comparison, we also calculated values for the 14 traits previously defined for C-BARQ data (subsequently referred to as "SetB traits") [18, 19], for the same data as in SetA.

Table 1. Description of the 12 SetA personality traits analysed in the study.

Trait	Pedigree analysis		Genomic analysis	
	Range	No. observations	Range	No. observations
Agitated	1 - 5	1901	1 - 5	780
Attention	1 - 5	1942	1 - 5	792
Barking	1 - 5	1955	1 - 5	795
Excitability	1 - 5	1962	1 - 5	777
Fetching	1 - 5	1953	1 - 5	798
HOFear	0.7 - 5	1970	0.73 – 3.33	776
NoiseFear	1 - 5	1942	1 - 5	788
NOAggression	1 – 3.86	1971	1 – 3.86	802
OAggression	1 – 2.43	1967	1 – 2.14	801
SepAnxiety	1 - 3	1947	1 – 2.75	856
Trainability	1 – 5	1969	2 – 5	799
Unusual	1 – 3.55	1968	1 – 3.55	800

Demographic factors

Factors included as fixed effects and covariates in the mixed linear animal models were based on information on management and physical traits recorded from a separate questionnaire sent to the dog owners [20, 21]. The fixed effects included sex and neuter status, housing, coat colour, health status, exercise per day and "Role" (based on the activities of the dog), as described in Table 2. The latter was determined using a stringent criterion such that in case of uncertainty, the value was recoded as missing. The age of the dog in days (760 – 3,380 days) was fitted as a covariate. Thus, seven demographic factors were fitted in the models (Table 2). All of these factors were shown to be associated with one or more traits in the previous analysis (Lofgren et al., 2014). Records with missing values (either trait values or fixed

effects) were removed from the analyses, thus resulting in variable numbers of observations for each trait.

Table 2. Description of factors included as fixed effects in genetic models. These include sex and neuter status (four levels), housing (three levels), coat colour (three levels), health status (two levels: healthy or having had some health problem during their lifetime), exercise per day (four levels) and "Role" (three levels).

Factors	**Categories**	**No. observations**
Coat colour	Black	1144
	Yellow	521
	Chocolate	310
	missing	0
Exercise per day	<1	315
(hours)	1-2	972
	2-4	565
	>4	118
	missing	5
Health	Some health problem during lifetime	1697
	No health problems	278
	missing	0
Housing	Primarily inside	1578
	Both inside and outside	170
	Primarily outside	176
	missing	51
Role	Gundog	840
	Pet	817
	Showdog	140
	missing	178
Sex/neutered status	Male entire	451
	Male neutered	59
	Female entire	1028
	Female neutered	426
	missing	11

Mixed linear models analysis

The pedigree used in the analysis was spread over 29 generations and included 28,943 dogs: 9,040 sires (from 3,837 paternal grand-sires and 6,524 paternal grand-dams) and 17,975 dams (from 6,555 maternal grand-sires and 12,272 maternal grand-dams). Approximately 70% of

148 the sires had only one offspring with phenotypes. The maximum number of phenotyped
149 offspring per sire was 37 (for one sire).

150 *Univariate Analysis*

151 For both SetA and SetB traits, the estimation of the variance components, heritability and
152 significance of fixed effects was carried out by fitting mixed linear models in ASReml [22].
153 The mixed linear models can be described as:

$$\boldsymbol{y} = \boldsymbol{X\tau} + \boldsymbol{Zu} + \boldsymbol{e}$$

154 Where $\boldsymbol{y}$ is the vector of observations, $\boldsymbol{\tau}$ is a vector of fixed effects, **X** is an incidence matrix
155 referring the observations pertaining to fixed effect levels described further below, $\boldsymbol{u}$ is a
156 vector of breeding values treated as random effects, $\boldsymbol{Z}$ is an incidence matrix referring
157 observations to their corresponding random effects, and $\boldsymbol{e}$ is a vector of residual effects,
158 assumed to be normally distributed according to the distribution $N(0, \sigma_e^2 \boldsymbol{I})$, where σ_e^2 is the
159 residual variance and $\boldsymbol{I}$ is the identity matrix.

160 The direct additive genetic effect of the dogs was fitted as the only random effect. In the
161 animal model, the vector of random effects $\boldsymbol{u}$ is assumed to be normally distributed according
162 to the distribution $N(0, \sigma_A^2 \boldsymbol{A})$, where σ_A^2 is the additive genetic variance and **A** is a numerator
163 relationship matrix. The heritability was estimated as:

$$h^2 = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_e^2}$$

164 The choice of effects included in the best fitting model was based on their p-value. The
165 model was constructed through backward elimination, i.e. by first fitting all effects, followed
166 by stepwise subtraction of the term with highest p-value from the model. Model construction
167 was performed separately in each trait, being carried out until all effects included were
168 significant. Thus, the final model was defined as the most comprehensive model in which all
169 fixed effects and covariates had a p-value below 0.05.

170 *Bivariate analysis*

171 Genetic and environmental correlations between SetA traits with h^2>0.2 were obtained by
172 fitting bivariate models to their records. The general model behind bivariate analyses is
173 similar to that presented in univariate analyses, but with $\boldsymbol{u}$ assumed to be MVN(0,$\boldsymbol{V} \otimes \boldsymbol{A}$),
174 where $\boldsymbol{V}$ is a (co)variance matrix of the two trait terms. The fixed effects fitted to each trait in

the bivariate analyses were the same as those fitted in the final model derived for each trait in the univariate analyses. The phenotypic, genetic and environmental correlations were calculated as:

$$r = \frac{cov_{XY}}{\sqrt{var_X var_Y}}$$

Where cov_{XY} is the covariance between the particular components of traits X and Y, and var_X and var_Y are the given variance components.

Bivariate analyses were also conducted between SetA and SetB traits for which a significant genetic variance was detected in the univariate analyses.

SNP genotyping and marker quality control

The genomic data was collected as part of a larger project [21, 23] where genotypes were obtained using the Illumina Canine High Density Beadchip containing 173,662 SNPs (http://www.illumina.com/documents/products/datasheets/datasheet_caninehd.pdf; accessed 27/04/16). Extraction of DNA from buccal swabs was performed according to standard protocols. DNA was resuspended in water and quantified using a Nanodrop and stored at 4°C until use. Filtering criteria have been previously applied to samples based on call rate and excessive genotyping errors [21]. Of the 1,179 animals that satisfied these quality control criteria, 885 were included in the set of 1,975 with C-BARQ assessments and thus were retained for the current study. Filtering criteria have also been previously applied to markers [21]. Using Genome Studio software (http://www.illumina.com/techniques/microarrays/array-data-analysis-experimental-design/genomestudio.html; accessed 27/04/16), 59,260 markers were discarded due to low call rate (<98%), low reproducibility (GTS < 0.6) and low or confounded signal (ABR mean < 0.3). Further quality control was applied using PLINK [24], removing SNPs on the sex chromosomes and those deviating from Hardy-Weinberg equilibrium (threshold of p<4.48E-7 applying a Bonferroni correction). Additional quality control involved the removal of markers with low minor allele frequency (MAF < 0.01) in the dataset of 885 dogs. The final set of 103,623 SNPs were assigned genomic positions according to the CanFam 2.0 assembly.

Genomic analyses

Genome-wide association analyses of the SetA traits were performed using GEMMA [25], accounting for population stratification by fitting the genomic relationship matrix (GRM, **G**). The linear mixed models were assumed as follows:

$$\boldsymbol{y}=\boldsymbol{W\alpha}+\boldsymbol{x}\beta+\boldsymbol{u}+\boldsymbol{e},$$

where $\boldsymbol{y}$ is the vector of phenotypes, $\boldsymbol{W}$ is the matrix of covariates with the $\boldsymbol{\alpha}$ vector of associated fixed effects (including the intercept) and $\boldsymbol{x}$ is the vector of marker genotypes (coded as 0/1/2) with β representing the regression coefficient of the marker genotype on the phenotype. The vectors of random polygenic effects, $\boldsymbol{u}$, and residual errors, $\boldsymbol{e}$ follow multivariate normal (MVN) distributions given by $\boldsymbol{u} \sim \text{MVN}(0, \sigma_g^2\ \mathbf{G})$ and $\boldsymbol{e} \sim \text{MVN}(0, \sigma_e^2\mathbf{I})$, where σ_g^2 and σ_e^2 are the variances associated with random polygenic ($\boldsymbol{u}$) and residual ($\boldsymbol{e}$) terms , respectively. Fixed effects were determined for each trait separately, based on results from the pedigree-based analysis (described above), with minor changes in coding. Thus, effects used were: sex (2 degrees of freedom, df), neuter status (2 df), Role (2 df, Gundog and Pet/Showdog) and exercise (1 df). Animals for which one or more fixed effects or covariates were missing were removed from the analysis, such that the number of animals included in the analysis varied across the traits (range: 778-878; analyses of nine of the 12 traits incorporated 802-807 animals) (Table 1).

The statistical significance for each marker was assessed using a Wald t-test. Due to the possibility of inflation of –log(p) as a result of differences in allele frequencies (cryptic population stratification) or genotyping errors, a correction to the p-values by the inflation factor λ was also performed using the method suggested by Amin et al. [26] under the assumption that the inflation is roughly constant across the genome. Following Bonferroni correction for multiple testing resulting from the large number of markers, significance thresholds (based on the corrected p-values) were $p< 4.825E-7$ for genome-wide ($p<0.05$) and $p<9.650E-6$ for suggestive (one false positive per genome scan) levels.

Estimations of the variance explained by the full set or subsets of SNPs were performed in GCTA [27, 28] using the same models as for the GWAS.

Results

Mixed linear models

The number of significant demographic factors affecting a personality trait differed between the SetA traits, ranging from just one significant effect detected for Barking to five effects detected for Unusual (Table 3). The factors with largest impact on personality traits were Role (11 traits) and sex-neuter status (8 traits). Exercise levels and coat colour were also associated with several traits (5 and 4 traits, respectively). Health status, housing and age were associated with the fewest traits (2, 2 and 1, respectively). Analysis of the SetB traits showed similar results, with sex-neuter status, Role and exercise levels having effects on the largest number of traits (Table 3).

248 Table 3. Summary of fixed effects and covariates found to be significantly ($p<0.05$) associated with
249 personality traits using mixed linear models.

Trait	Factor						
SetA	Age	Coat colour	Gender/ Neuter	Health	Housing	Exercise	Role
Agitated		√					√
Attention			√				√
Barking							√
Excitability					√	√	√
Fetching	√	√					√
HOFear			√		√		√
NoiseFear			√				√
NOAggression			√	√		√	√
OAggression			√				√
SepAnxiety		√	√	√		√	
Trainability			√			√	√
Unusual		√	√			√	√
SetB							
Attachment			√			√	√
Chasing			√			√	√
Dog-directed aggression						√	√
Dog-directed fear			√				√
Dog rivalry							
Energy level	√	√	√				
Excitability					√	√	√
Non-social fear			√				√
Owner-directed aggression						√	√
Separation-related behavior			√	√		√	
Stranger-directed aggression			√			√	√
Stranger-directed fear			√				
Touch sensitivity						√	√
Trainability						√	√

250

The h^2 estimates from the best-fitting models for the SetA traits varied from 0.03 (SE 0.04) for OAggression to 0.38 (SE 0.08) for Fetching (Table 4). Heritabilities greater than 0.20 were found for six traits (shown in Table 4 in bold). All traits except OAggression and SepAnxiety were found to have genetic variance significantly greater than 0.

Table 4. Pedigree-based (SetA and SetB) and genomic (SetA) heritability estimates and associated standard errors for trait-specific models (fixed effects and covariates fitted as shown in Table 2). Values ≥ 0.20 shown in bold.

Trait (SetA) (number of questions on which it was based)	h^2 (SE)	genomic h^2 (SE)	Number of questions in common	Traits (SetB) (number of questions on which it was based)	h^2 (SE)
Agitated (2)	**0.22 (0.07)**	0.02 (0.03)	2	Attachment (6)	0.13 (0.06)
Attention (3)	0.14 (0.06)	0.00 (0.05)	3		
Barking (1)	0.15 (0.07)	0.10 (0.07)			
Excitability (5)	0.10 (0.06)	0.00 (0.05)	5	Excitability (6)	0.11 (0.06)
Fetching (1)	**0.38 (0.08)**	0.18 (0.08)			
HOFear (15)	0.08 (0.05)	0.13 (0.06)	4	Stranger-directed fear (4)	0.14 (0.06)
			4	Dog-directed fear (4)	0.07 (0.05)
NoiseFear (2)	**0.30 (0.08)**	**0.23 (0.07)**	2	Non-social fear (6)	**0.25** (0.08)
NOAggression (14)	**0.29 (0.08)**	**0.20 (0.07)**	8	Stranger-directed aggression (9)	**0.26** (0.07)
			4	Dog-directed aggression (4)	0.17 (0.07)
OAggression (7)	0.03 (0.04)	0.05 (0.06)	7	Owner-directed aggression (8)	0.02 (0.03)
SepAnxiety* (8)	0.06 (0.05)	0.00 (0.04)	8	Separation-related behaviour* (8)	0.00 (0.02)
Trainability (7)	**0.28 (0.07)**	**0.20 (0.07)**	7	Trainability (8)	0.15 (0.06)
Unusual (20)	**0.25 (0.08)**	0.11 (0.07)	3	Chasing (4)	**0.26** (0.07)
				Dog rivalry (4)	0.11 (0.06)
				Energy level (2)	0.15 (0.06)
				Touch sensitivity (3)	0.18 (0.08)

* These two traits had the same definition but heritability estimates were slightly different due to different rules regarding treatment of missing values for individual CBARQ responses.

The range of heritability estimates for the SetB traits were somewhat lower than for the SetA traits (Table 4), with similarities between some related traits (e.g. NoiseFear and Non-social Fear, NOAggression and Stranger-directed aggression, Unusual and Chasing) but also some notable differences (e.g. SetA_Trainability greater than SetB_Trainability).

Only six out of 44 of the SetA trait pairs were found to be significantly genetically correlated (Supplementary Table S1; the genetic correlation for NoiseFear-HOFear could not be estimated due to a singularity in the average information matrix computed by the ASREML algorithm). Four of these involved Unusual Behaviour (with Agitated, NoiseFear, NOAggression and Trainability). The other significant genetic correlations involved NOAggression (with Fetching and HOFear). The significant correlations were mostly moderate and positive, with the exception of that between Unusual and Trainability. In contrast, more than half of the residual correlations (28 out of 44) between the SetA traits were found to be significant, suggesting shared environmental influences. The residual correlations varied in sign and magnitude, with the strongest negative correlation found for Trainability and Unusual (r_e= -0.36, SE 0.06) and the strongest positive correlation found for Excitability and Unusual (r_e= 0.42, SE 0.05).

Genetic correlations between SetA and SetB traits are given in Supplementary Table S2 (the analysis failed for the NoiseFear (SetA) - Non-social Fear (SetB) pair due to a singularity in the average information matrix computed by the ASREML algorithm). For some related trait pairs, the genetic correlation was very high (e.g. SetA-SetB: Excitability-Excitability, r_g=0.98, SE 0.01; NOAggression-Stranger-directed-aggression, r_g =0.98, SE 0.03) while it was not as high for others (e.g. Trainability-Trainability, r_g =0.55, SE 0.18). Another notable estimate was between Unusual (SetA) and Chase (SetB) (r_g =0.88, SE 0.07).

Genomic Analyses

The proportion of the phenotypic variance explained by the full set of SNPs ("genomic heritabilities"), based on a smaller dataset than that of the pedigree-based heritabilities, ranged from 0.00 (Attention, Excitability, SepAnxiety; SE ~0.04) to 0.23 (NoiseFear; SE 0.07) (Table 4). Ten of the traits showed lower genomic heritabilities than the pedigree-based estimates; for the majority of these traits, the SNP data explained less than half of the pedigree-based heritability, although for two traits (Trainability and NoiseFear), the SNP data

explained >70% of the pedigree-based heritability. For HOFear and OAggression, the genomic heritabilities were higher than the pedigree heritabilities, although the differences were not significant.

GWAS detected one genome-wide significant SNP for SepAnxiety (CFA3:94,526,955, β=0.3279, SE 0.06081) (Figure 1). We also identified 27 SNPs (in 16 genomic regions) showing suggestive significance ("suggestive SNPs") for eight out of the twelve SetA traits: Agitated (CFA18), Barking (CFA4), Fetching (CFA1, 4 and 22), NoiseFear (CFA20), NOAggression (CFA9), OAggression (CFA5, 14, 28 and 31), SepAnxiety (CFA3, 20) and Unusual (CFA2) (Table 5; Supplementary Figure 1). A visual inspection of Quantile-Quantile (Q-Q) plots revealed that the lambda-correction procedure adequately corrected for unexplained population structure in the sample (Supplementary Figure 2).

The genomic region bounded by the significant and suggestive SNPs on CFA3 explained ~0.03 of the phenotypic variance of SepAnxiety (approximately half of the pedigree-based heritability), despite its estimated genomic heritability of 0.00. However, the significant SNP has a very low minor allele frequency (0.01) and the minor homozygote is absent (also true for the other two SNPs in this region), which may have compromised the estimate of its effect size. The proportion of the variance explained by the individual suggestive SNPs across the genome ranged from 0.022 to 0.041 across the traits (Table 5).

Figure 1. Results from genome-wide association analysis of SepAnxiety. A. –log(p) values for all SNPs across the genome. The genome-wide threshold (red line) corresponds to the Bonferroni correction for a nominal P-value = 0.05. The suggestive threshold (blue line) corresponds to one false positive per genome scan. B. Q-Q plot of Expected versus Observed p-values.

Table 5. SNPs exceeding suggestive level threshold in genome-wide association analysis (SNP showing genome-wide significance shown in **bold**).

Trait	Chrom	Position*	SNP	Effect size (β)[¥] (SE)	Corrected p-value	Proportion of variance explained[§]
Agitated	18	50359100	BICF2P964118	-0.2541 (0.05)	2.22e-06	0.029
Barking	4	55645061	BICF2P696817	-0.2251 (0.05)	8.52e-06	0.029
Fetching	1	84905345	BICF2G630792579	-0.2925 (0.06)	6.60E-06	0.029
	4	91287944	BICF2P844921	-0.3267 (0.07)	9.58E-07	0.031
	4	91442298	BICF2P456276	-0.3600 (0.08)	1.91E-06	0.029
	4	91453025	BICF2P73495	-0.3627 (0.08)	1.99E-06	0.029
	4	91475109	BICF2P519369	-0.4005 (0.09)	4.19E-06	0.026
	22	35218609	BICF2S2314224	-0.6586 (0.15)	7.01E-06	0.023
NoiseFear	20	31482825	BICF2P846231	0.3961 (0.09)	5.86e-06	0.028
NOAggression	9	28762604	BICF2G630832223	-0.1212 (0.03)	8.67e-06	0.027
OAggression	5	19381324	BICF2S2362330	0.1174 (0.03)	4.98E-06	0.025
	5	19420165	BICF2P935231	0.08785 (0.02)	1.30E-06	0.030
	5	39447402	BICF2G630184924	0.06829 (0.01)	1.94E-06	0.030
	5	41496464	BICF2G630186215	0.05384 (0.01)	5.09E-06	0.027
	5	41528401	BICF2G630186251	0.0530 (0.01)	6.61E-06	0.026
	5	41575639	BICF2G630186301	0.05375 (0.01)	4.79E-06	0.027
	5	41596934	BICF2G630186303	0.05247 (0.01)	7.32E-06	0.026
	5	41690223	BICF2G630186310	0.05613 (0.01)	2.43E-06	0.028
	14	22804558	BICF2P319167	0.1186 (0.03)	6.86E-06	0.024
	21	45731572	BICF2P1339075	0.1437 (0.03)	1.36E-06	0.041
	28	11671986	BICF2S23541632	0.0732 (0.02)	6.71E-06	0.025
	31	32326939	BICF2G630739766	0.0932 (0.02)	8.72E-06	0.023
SepAnxiety	3	93609499	BICF2P186901	0.2848 (0.06)	2.75E-06	0.023
	3	94469321	BICF2S2323991	0.2847 (0.06)	2.76E-06	0.023
	3	**94526955**	**BICF2G630362033**	0.3279 (0.06)	**2.52E-08**	**0.033**
	20	15556881	BICF2P1395346	0.1840 (0.04)	3.81E-06	0.022
Unusual	2	77975665	BICF2P612229	0.3294 (0.07)	6.65e-06	0.027

*SNP positions according to CanFam2.0

¥ Additive effect of the minor allele

§ Proportion of variance explained: $2pq\beta^2/\sigma^2_P$, where p (q) = minor (major) allele frequency, σ^2_P = phenotypic variance (these values are very similar to those estimated using GCTA)

Discussion

The analysis of C-BARQ answers collected from owners of Labrador Retrievers in the UK revealed a significant genetic variance present for most of the behavioural traits examined. The magnitude of the estimates significantly different from 0 for the SetA traits ranged between 0.08 (HOFear) and 0.38 (Fetching), showing consistency with the range of heritabilities previously reported for behavioural traits in dogs [4, 29-31] (see also review by [32]). For most traits, genomic heritabilities were considerably lower than pedigree-based estimates, however, genome-wide association analysis identified several genomic regions showing suggestive associations with C-BARQ traits. While C-BARQ has been used in a large number of studies on dog behaviour, the genetic analysis of the traits derived from the questionnaire is still in its infancy, with only a handful of heritability estimates published to date (e.g. [15, 16]). The results presented in this study show that there is a consistency in detection of the genetic variance and detectable genomic associations for traits derived from C-BARQ, but also that quantification of the genetic component of C-BARQ-based traits is sensitive to how these behavioural factors are extracted from the questionnaire responses.

Heritability estimates and trait definition for C-BARQ data

The SetA traits with highest heritability were Fetching and NoiseFear. Our estimate for the latter falls within the range of previous reports based on standardised tests, with heritabilities of "reaction to gunfire" ranging between 0.23 and 0.56 [2, 33]. The heritability estimate for Non-social fear (SetB) was similar to NoiseFear for this dataset and somewhat lower than found previously for Rough Collies (h^2=0.36, SE 0.06) [16]. Thus it appears that genetic variation for this trait exists in various breeds, including gun dogs.

Fetching was only considered as a separate trait for SetA. In SetB, the question related to fetching ability was included in Trainability (h^2=0.15, SE 0.06). Treating Fetching and Trainability as separate traits resulted in higher heritability estimates for both: h^2=0.38 (SE 0.08) for Fetching and h^2=0.28 (SE 0.07) for Trainability, with a positive but small genetic correlation between the traits (r_g=0.26, SE 0.18). Heritabilities for Trainability (SetB) have been previously estimated at 0.15 (SE 0.04) for Rough Collies [16] and 0.25 (SE 0.04–0.06) across 14 breeds (not including either Labrador Retrievers or Rough Collies) [29]. The genetic correlations between SetA and SetB traits demonstrate the large influence of fetching ability on SetB Trainability for this population such that they are higher for Fetching (SetA) –

Trainability (SetB) (r_g =0.78, SE 0.11) than for Trainability (SetA) – Trainability (SetB) (r_g =0.55, SE 0.18). These results suggest, at least in Labrador Retrievers, some degree of distinction between the genetic basis for fetching ability and other trainability characteristics.

Agitated and Attention were considered as separate traits in SetA but together contributed to Attachment in SetB. The heritability estimate for Attention (SetA) was very similar to that of Attachment (SetB), with a high genetic correlation (r_g=0.86, SE 0.08). The estimate of heritability for Agitated (SetA) was higher than the estimate for Attachment (SetB), with a lower genetic correlation (r_g=0.62, SE 0.17). These results suggest that there may be differences between the genetic influences on Agitated and Attention.

In contrast to the above-mentioned traits, Unusual (SetA) was constructed from a much larger number of questions (20) than any of the SetB traits. The significant genetic correlations between Unusual and several other SetA traits confirm the multidimensionality of this trait. Its estimate of heritability of 0.25 (SE 0.08) invites further investigation, as the questions incorporated in this trait cover a wide range of behaviours and not all were expected to share genetic variance. However, several of the questions that are included in Unusual and show substantial variation between dogs refer to chasing behaviours, thus the genetic variance may largely reflect these characteristics. This is supported by a large, significant and positive genetic correlation between Unusual and Chasing (SetB) (r_g=0.88, SE 0.07).

Moderate heritability estimates were found for several other SetA traits, including NOAggression, which included questions related to aggression towards unfamiliar humans as well as dogs. Its heritability was very similar to that for Stranger-directed aggression (SetB) and further, the two traits showed a high genetic correlation, not significantly different from 1 (r_g=0.98, SE 0.03). The C-BARQ questions relating to aggression, particularly aggression directed toward strangers, show good consistency across studies [16, 19, 34]. Aggressive behaviours in dogs have been shown to fall into different categories based on the target, e.g. owner, child, stranger or dog [35]. Moderate heritability detected for NOAggression and Stranger-directed Aggression are similar to estimates for Stranger-directed Aggression in Rough Collies (h^2=0.24, SE 0.05) [16] and other breeds (h^2~0.21, SE not given) [29]. In contrast to aggression directed towards strangers and other dogs, our estimate of heritability for owner-directed aggression was not significantly different from 0, in accordance with previous reports showing low or no genetic variance, most likely due to strong selection intensity against this trait, particularly in breeds of large size [29, 36].

While the questions contained in the C-BARQ questionnaire seem to capture the variance of the behavioural traits, the method of grouping into behavioural factors may influence estimates of heritability, as was shown above for Trainability and also suggested for Agitated. One alternative approach to trait definition could involve grouping questions based on their genetic, rather than phenotypic, covariances. Such an approach has been shown in the context of standardised behavioural tests to improve the estimates of the behavioural dimensions of the temperament test used by the Swedish Armed Forces, especially when items with 0 genetic variance were removed from the factor [3]. Evaluating the genetic variance of individual C-BARQ questions has only been carried out once to our knowledge, based on data for young (6 and 12 months old) guide dog candidates [37]. Using a similar approach, it would be interesting to examine the heritabilities of particular questions, as well as their genetic correlations, using data collected from adult dogs.

In considering how to interpret results of genetic studies on behavioural traits, it is important to recognise that dog breeds may differ in terms of the meaningfulness (and thus heritability) of behavioural constructs, as is suggested by differences between heritability estimates for Labrador Retrievers (our study) and Rough Collies [16], which could be due to differences in breed history or the intensity of selection for specific traits. Depending on the scientific question or practical application, researchers may need to make a choice between using the same trait definitions across breeds but accepting that their meaning differs between breeds or alternatively, developing breed-specific trait definitions that show similar levels of genetic variation.

Along with illustrating how trait definition may influence estimates of genetic variance, results from this study emphasize the important role of lifestyle and management factors on behavioural traits. Due to the strong associations with these factors, it would be prudent in the future to develop a standardized questionnaire detailing the possible sources of the environmental effects on the dog's behaviour; this could accompany the C-BARQ questionnaire and would allow a more standardized decomposition of the trait variance across populations.

Genomic regions showing associations with personality traits

The limited number of molecular genetic studies of canine behaviour mainly comprise candidate gene studies or studies targeted at clinical behavioural disorders, which tend to

have more clearly defined phenotypes than everyday life behaviours. The few studies using genomic techniques to address everyday life behaviour have primarily implemented between-breed comparisons based on breed-average phenotypes (e.g. [38, 39]). This approach has limitations in that behavioural and physical traits distinguishing breeds are often confounded, making it difficult to identify which trait is associated with a particular genomic region. Analysis of within-breed genotypic and phenotypic variation avoids this problem although the variants (genes) that contribute to behavioural differences within breeds may not be the same as those that account for between-breed behavioural variation.

Based on results in mice, behavioural traits are suspected to be largely polygenic, with a strong environmental component [7, 40], thus, difficulties are expected in detecting genomic associations. Our results were consistent with a model of polygenic inheritance for most traits, nevertheless, one significant association and several suggestive associations were identified, albeit only explaining small proportions of the phenotypic variance. Based on the shape of the GWAS peaks (i.e. the number of suggestive SNPs within the identified regions), the most convincing genomic associations were identified for Fetching (CFA4) and OAggression (CFA5). The largest effect sizes were seen for Fetching (CFA4 and CFA22) and NoiseFear (CFA20).

Although SepAnxiety showed very low genetic variance, the genomic analyses indicated a significant association with the CFA3 region, which may be due to limitations of heritability estimation if the assumptions of the infinitesimal model are not met and the effect size is small. Alternatively this discrepancy may reflect a problem of estimation of effect size for such a rare haplotype, i.e. a false positive association. A similar situation may apply to OAggression, for which the estimated heritability was not significantly greater than 0 but several markers on CFA5 showed suggestive associations; however, minor allele frequencies for these markers (4-6%) were greater than those associated with SepAnxiety, such that estimates of effect sizes will have been more accurate. Additional data will be required to confirm and resolve the identified genomic associations.

Several SNPs showing suggestive or significant associations with the CBARQ traits were found close to genes with known neurological or behavioural functions. The *TH* (tyrosinase hydroxylase) gene, whose enzyme product is involved in the synthesis of L-DOPA, the precursor of the neurotransmitter dopamine, is located ~1 Mb from the SNP on CFA18 associated with Agitated. Dopamine plays numerous functions and several distinct dopamine

pathways are found in the brain. Furthermore, conditions in humans involving inattention and impulsivity, such as attention deficit hyperactivity disorder (ADHD), are associated with decreased dopamine activity [41]. Polymorphism in *TH* has previously been associated with activity, impulsivity and inattention in two dog breeds [9, 11]. Studies have also shown an association between *TH* polymorphisms in humans and "neuroticism" (tendency to experience negative emotions) and "extraversion" (characterized by sociability and excitability) [42, 43], two personality traits associated with impulsivity [44].

Genes in the suggestive or significant GWAS peak regions on CFA3, CFA4 and CFA20 have also been associated with neurological functions. *CPLX1*, which encodes complexin 1, is located in the CFA3 region associated with SepAnxiety (~49 kb from the genome-wide significant SNP). Complexins are cytoplasmic neuronal proteins that regulate neurotransmitter release [45]. Complexin genes have been proposed as candidate loci for associations with psychiatric disease due to their neurobiological functions [46] and knock-out mice lacking complexins have been shown to exhibit unusual behaviours [47, 48]. The SNP on CFA4 associated with Barking is located ~5 kb from *CLINT1* (*Epsin 4*), a gene for which mutations have been associated with susceptibility to schizophrenia [49]. Finally, the SNP associated with NoiseFear is located ~0.27 Mb from *CADPS2* on CFA20. *CADPS2* is a member of a gene family encoding calcium binding proteins that regulate the exocytosis of neuropeptide-encompassing (dense-core) vesicles from neurons and neuroendocrine cells. The gene and its variants have been associated with autism in humans [50, 51] and with various behavioural and neurological phenotypes in mice [52]. An association with noise phobia on CFA20 (position not given) was previously reported for dogs [53].

Conclusions

The analysis of an owner-evaluated behavioural questionnaire, C-BARQ, together with a questionnaire examining demographic factors, revealed significant genetic variation for most of the behavioural traits studied in a population of Labrador Retrievers. While C-BARQ questionnaires are thus confirmed as a valuable tool in detecting genetic variance in everyday life behaviours of dogs across different lifestyles, it has been shown that the grouping of the questions into behavioural factors may have a considerable effect on the magnitude of the genetic variance detected. Further work is needed to devise the optimal method of extracting information about the genetic background of the behaviours from the questionnaire

responses. A model of polygenic inheritance with small effect sizes is consistent with most traits investigated in this study. Chromosomal regions associated with some traits were suggested by genomic analyses, however, additional data will be required to confirm and resolve the genomic associations.

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Supplementary material

Figure S1. Results from genome-wide association analysis of all 12 personality traits: -log(p) values for all SNPs across the genome. The genome-wide threshold (red line) corresponds to the Bonferroni correction for a nominal P-value = 0.05. The suggestive threshold (blue line) corresponds to one false positive per genome scan.

Figure S2. Results from genome-wide association analysis of all 12 personality traits: Q-Q plot of Expected versus Observed p-values.

Table S1. Results from bivariate analysis for pairs of SetA traits with significant genetic variance determined by univariate analyses (the genetic correlation for NoiseFear-HOFear could not be estimated, see text); factors included in the model were the same as those fitted in the final models derived for each trait in the univariate analyses (see Table 4). Above diagonal: additive genetic correlations (standard errors); below diagonal: residual correlations (standard errors). Those shown in bold are significantly greater than 0 ($p<0.05$).

Table S2. Genetic correlations between SetA and SetB traits with significant genetic variance determined by univariate analyses (the genetic correlation for NoiseFear-Non-social Fear could not be estimated, see text); factors included in the model were the same as those fitted in the final models derived for each trait in the univariate analyses (see Table 4). Those shown in bold are significantly greater than 0 ($p<0.05$).

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